REMARKS

The claims have been amended in accordance with the discussion at the interview. Claim 1 has been amended to incorporate the editorial suggestions kindly provided by Dr. Weber; step (b) has been clarified as an identifying step and a step for recovery has been added in view of the preamble reflecting "producing" a soluble protein. All independent claims require this step as the object of the invention is to provide a domain suitable for 3-dimensional analysis. It has also been clarified that the nucleotide sequences encode different fragments of the starting protein.

Claims 3 and 19 have been amended to delete the unnecessary phrase "and functional portions thereof." If an amino acid sequence is functional, it is behaving as an enzyme, a binding protein, a luminescent protein or a fluorescent protein regardless of its origins. Thus, the phrase "and functional portions thereof" is believed unnecessary. Claims 4, 7, 20, 21 and 22 have been amended to clarify that variants of GFP are indeed "GFP variants." This change was also agreed to at the interview. Claims 5 and 7 have also been amended for clarification only; applicants realize that claim 7 should refer to the nucleotide sequences encoding the fusion proteins being expressed and have changed "selecting" to "identifying."

As further agreed at the interview, claims 11 and 12 were canceled as redundant with other claims.

Claim 13 has been amended in a similar manner to claim 1. Also, in part (b), it was recognized that the fragment of DNA encoding the "first" protein was the correct referent.

Claim 14 has been amended in a manner similar to claim 5. Claims 16-18 have been amended to clarify the referent of "preparing."

The amendments conform to those discussed and agreed upon at the interview and are for clarification. No new matter has been added and entry of the amendment is respectfully requested.

The Invention

As set forth in the specification, the invention is directed to a method to identify soluble protein domains that are suitable for three dimensional structural analysis (page 3, lines 18-20). As pointed out on page 11, the synthesized soluble domains of the proteins can be used for 3-dimensional structural analyses. This provides a simpler substrate for analysis than the entire protein. The soluble domains can be analyzed individually with many fewer variables in an exercise which is in itself quite complex. Thus, the object of the invention is not to obtain a soluble protein *per se*, but rather to provide a smaller portion of a protein that is suitable for X-ray analysis. In order for this to be the case, however, this domain must itself be soluble.

The invention accomplishes this by taking advantage of the fact that when a soluble amino acid sequence is included in a fusion protein with a functional protein, the functional protein retains its function. On the other hand, if the amino acid sequence included is not soluble, the function of the fused protein is destroyed or greatly reduced. Thus, at the crux of the invention is providing a fusion protein that contains various fragments of a protein as candidates for soluble domains which, when identified, can be synthesized for use in determination of three dimensional structure. The invention does this by coupling fragments to functional proteins and identifying as a successful fragment that fragment whose fusion remains functional. The various independent claims are directed to various levels of generality of this principle.

Thus, claim 1 requires the recovery of the synthesized soluble domain and recombinant expression of the fusion proteins that will be identified. Claim 1 is, however, silent at to the nature

of the function exhibited by the protein included in the fusion and also with respect to the manner in which the fragments are provided.

Claim 10 is more specific in that it requires that the function be that of green fluorescent protein or a GFP variant and describes the manner in which the fragments are prepared (using a DNA digesting enzyme).

Claim 13 does not specify the nature of the functional protein included in the fusion, but specifies digestion of the encoding DNA as the manner of obtaining the fragments.

Claim 15 is directed to a method to produce a soluble domain where synthesis and recovery are the only active steps. However, the soluble domain must have been identified by steps (i), (ii) and (iii).

Claim 21 specifies the manner of expression of the fusion proteins as well as requiring GFP or a GFP variant as the function. It is silent on the method of producing the fragments.

Should any unclarity remain as to the subject matter of the claims, a telephone call to the undersigned is respectfully requested.

The Objection to the Specification

Applicants have given consideration to the identification of what are asserted to be trademarks on pages 13 and 14 of the specification. However, in reviewing these designations, it is clear that the terms are not being used as trademarks, but rather as names of companies. Thus, capitalization and ™ would be inappropriate in this context.

It is believed that the objection to claim 16 is overcome by amendment and the discussion at the interview. Claim 15 is further limited by claim 16 because claim 15 does not require the preparing in step (i) to be in a cell-free system. It is also not inconsistent with claims 17 and 18.

Applicants are pleased that it was agreed at the interview that *E. coli* need be spelled out only in its first occurrence (in claim 6).

Rejection Under 35 U.S.C. § 112, First Paragraph

Applicants appreciate that the proposed amendments are considered to overcome the outstanding rejections under this section.

Specifically, claim 1 was criticized as lacking a recovery step; this has been addressed by amendment.

As to claim 3, applicants believe that a multiplicity of functional proteins are workable in the method of the invention and there is no evidence to the contrary. Applicants have outlined the nature of a number of activities for which assays are available and which could be used in the invention on page 8, line 1-page 9, line 14. The objected-to terms "functional portion" and "variant thereof" have been addressed by amendment.

As to claim 5, it is believed that the amendment clarifies the antecedent basis for the word "clone."

Claim 10 was criticized as lacking a recovery step, this has been addressed by amendment. The criticism of claim 11 is mooted by its cancellation.

As to claims 13, 15 and 21, the preamble and final step are matched in these cases as well.

Accordingly, these rejections may be withdrawn.

The Rejections Under 35 U.S.C. § 112, Second Paragraph

It is believed that the discussion at the interview and amendments to the claims for clarification dispose of this rejection. There are no essential steps missing from claim 1. The

criticized phrase "said starting protein that is a soluble domain" is incomplete – the complete statement reads "a fragment of said starting protein that is a soluble domain." There is antecedent basis for "soluble domain" of step (c) in step (a) as the Examiner recognizes. Similarly, "said function" in step (b) is preceded by "a function" in step (a) providing antecedent basis.

It is believed the Markush wording in claim 5 is correct and that the amendment to claim 5 clarifies antecedent basis.

In claim 10, "a" has been added by amendment. Claim 10 is limited to green fluorescent protein and thus the further criticism is not understood.

Claim 11 has been canceled.

Claim 13 has been amended to provide antecedent basis for "the function." Claim 15 has been amended to change "portion" to "fragment." It is believed that "each said fusion protein" is grammatically correct. It is believed clear that the starting protein is the protein that provides the fragment rather than the functional portion of the fusion. The indefiniteness with respect to the preamble has been remedied as suggested as the interview.

Claim 21 has been amended to conform the language to consistent usage.

It is believed these amendments are responsive to the rejections for indefiniteness and applicants appreciate that this was agreed upon at the interview.

<u>The Rejection Over Chien in Combination with Waldo</u>

Claims 1, 3-7, 10-16 and 19-20 were rejected as assertedly obvious over Chien in view of Waldo.

Applicants are gratified that this rejection is dropped based on the discussion at the interview. As noted, Chien is completely different from the method of the invention in that it is a

"two hybrid" system for assessing interaction of proteins intracellularly. A diagram comparing Chien with the invention method is set forth on page 14 of the previous response and reference may be made to this diagram if necessary. However, applicants are gratified that this rejection will be withdrawn based on further understanding of the nature of the invention.

The Rejection Over Waldo

Claims 10-14 and 21-22 were rejected as obvious over Waldo. Again, applicants are gratified that this rejection is overcome. Waldo uses green fluorescent protein fusions as an index to identify mutants that will fold correctly when prepared recombinantly. Waldo does not use a cell-free system to synthesize a soluble domain of a larger protein. That is the object of the present invention. The present invention uses a cell-free system to synthesize a soluble domain of a larger protein. Waldo synthesizes recombinantly intracellularly mutants of complete proteins that have modified amino acid sequences. Thus, although Waldo recognizes that maintaining the fluorescence of green fluorescent protein in a fusion protein indicates correct folding of its fusion partner, this is not employed to identify smaller domains of a larger protein, and no synthesis of any soluble fragment is conducted in a cell-free system. Waldo's contribution to the art is recognized in the specification on page 3.

Applicants appreciate the withdrawal of this basis for rejection, as indicated at the interview.

The Rejection of Claims 15-16 and 19-22 Over Kawasaki

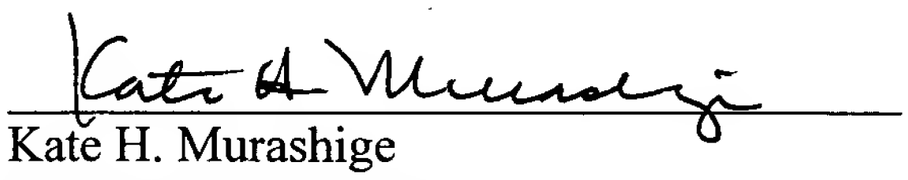
Kawasaki employs PCR to synthesize DNA encoding random portions of Vav protein and ligated these DNA's into a vector such that a fusion protein encoded by the GFP and the amplified DNA from the Vav protein would be produced. Those *E. coli* that maintained fluorescence were

CONCLUSION

Applicants wish, again, to express their appreciation to Examiners Robinson and Weber for the helpful interview. It is believed that the discussion at the interview, the amendments to the claims, and the discussion herein as well as the declaration under 37 C.F.R. § 1.131 dispose of all outstanding rejections and claims 1, 3-7, 10 and 13-23 are in a position for allowance. Passage of these claims to issue is respectfully requested.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, applicants petition for any required relief including extensions of time and authorize the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket No. 251002009400.

Respectfully submitted,

Dated: December 22, 2004 By: 

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